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Non-nucleotide linking reagents for nucleotide probes.

Abstract:

A versatile reagent with a non-nucleotide monomeric unit having a ligand, and first and second coupling groups which are linked to the non-nucleotide monomeric unit. The ligand can be either a functional moiety, such as a label or intercalator, or a linking arm which can be linked to such a moiety. Such reagent permits preparation of versatile nucleotide/non-nucleotide polymers, having any desired sequence of nucleotide and non-nucleotide monomeric unit
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s, each of the latter of which bear a desired ligand. These polymers can for example, be used as probes which can exhibit enhanced sensitivity and/or which are capable of detecting a genus of nucleotides each species of which has a common target nucleotide sequence of interest bridged by different sequences not of interest.

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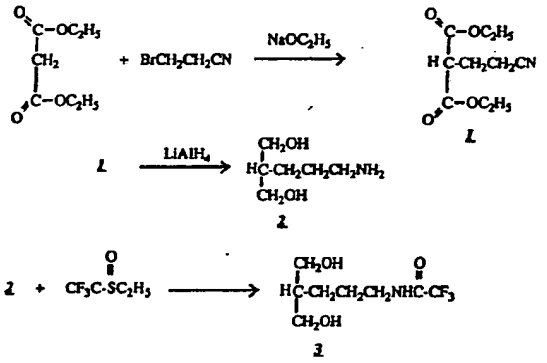
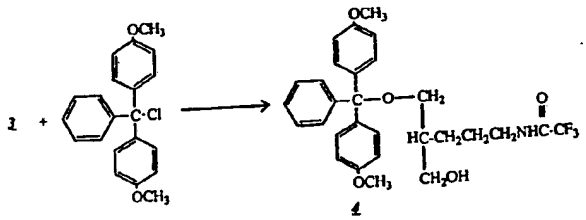
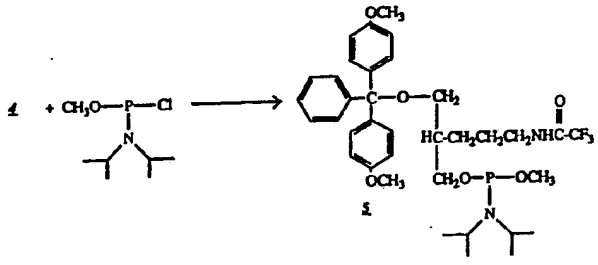
(54) Title: NON-NUCLEOTIDE LINKING REAGENTS FOR NUCLEOTIDE PROBES

(57) Abstract

A versatile reagent with a non-nucleotide monomeric unit having a ligand, and first and second coupling groups which are linked to the non-nucleotide monomeric unit. The ligand can be either a functional moiety, such as a label or intercalator, or a linking arm which can be linked to such a moiety. Such reagent permits preparation of versatile nucleotide/non-nucleotide polymers, having any desired sequence of nucleotide and non-nucleotide monomeric units, each of the latter of which bear a desired ligand. These polymers can for example, be used as probes which can exhibit enhanced sensitivity and/or which are capable of detecting a genus of nucleotides each species of which has a common target nucleotide sequence of interest bridged by different sequences not of interest.







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AMENDED CLAIMS

[received by the International Bureau on 7 April 1989 (07.04.89);
original claims 1,3,10,11,15,16,18,19,23,25,31 ,33-35 and 43 amended; new claims
49 and 50 added; other claims unchanged (16 pages)]

1. (amended) A non-nucleotide reagent suitable for preparing a nucleotide/non-nucleotide polymer, comprising:

(a) a non-nucleotide monomeric unit, having a ligand which is selected from a side arm with an attached chemical moiety, and from a linking arm which can be activated under non-adverse conditions so as to be capable of linking with a chemical moiety;

(b) first and second non-nucleotide coupling groups linked to the monomeric unit, the first coupling group of which is capable of coupling the non-nucleotide monomeric unit to a first additional monomeric unit, while the second coupling group remains inactivated so as to be substantially incapable of coupling, but can thereafter be activated under non-adverse conditions to couple the non-nucleotide monomeric unit to a second additional monomeric unit, at least one of the first and second additional monomeric units being a nucleotide monomeric unit.

2. A reagent as defined in claim 1, wherein said ligand is selected from a label, an intercalator, a metal chelator, drugs, hormones, proteins, peptide, haptens,

radical generators, nucleolytic agents, proteolytic agents, catalysts, specific binding substances, agents which modify DNA transport, and substances which alter nucleotide multimer solubility, and from a linking functional group which can be linked to any of the foregoing chemical moieties.

3. A non-nucleotide reagent suitable for preparing a nucleotide/non-nucleotide polymer, comprising:

(a) a non-nucleotide monomeric unit, having a ligand which is selected from a side arm with an attached chemical moiety, and from a protected linking arm which can be deprotected under non-adverse conditions so as to be capable of linking with a chemical moiety;

(b) a non-nucleotide, first coupling group and protected second coupling group, linked to the monomeric unit, the first coupling group which is capable of coupling the non-nucleotide monomeric unit to a first additional monomeric unit while the second one is substantially incapable of coupling, but can thereafter be deprotected under non-adverse conditions, so as to be capable of coupling the non-nucleotide monomeric unit to a second additional monomeric unit,

at least one of the first and second monomeric units being a nucleotide monomeric unit.

4. A reagent as defined in claim 3, wherein said ligand is selected from a label, an intercalator, a metal chelator, drugs, hormones, proteins, peptides, haptens, radical generators, nucleolytic agents, proteolytic agents, catalysts, specific binding substances, agents which modify DNA transport, and substances which alter nucleotide multimer solubility, and from a linking functional group which can be linked to any of the foregoing chemical moieties.

5. A reagent as defined in claim 4 wherein the first and second coupling groups, are capable of coupling the non-nucleotide monomeric unit to a 5' hydroxyl and a 3' phosphate of a monomeric unit, respectively.

6. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has an acyclic skeleton to respective ends of which the first and second coupling groups are linked.

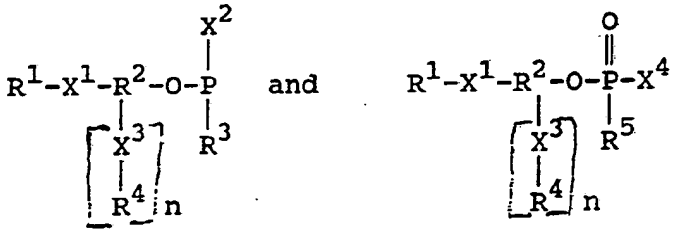
7. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has a skeleton to respective

ends of which the first and second coupling groups are linked, the skeleton being a 1 to 20 atom chain.

8. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has a skeleton to respective ends of which are linked the coupling groups, the skeleton being an acyclic hydrocarbon chain of from 1 to 10 carbon atoms.

9. A reagent as defined in claim 5 wherein the non-nucleotide monomeric unit has a skeleton to respective ends of which are linked the coupling groups, and wherein the linking arm is a 1 to 25 atom chain extending from the skeleton.

10. (amended) A reagent as defined in claim 3 selected from one of the compounds having the formula: wherein:



(a) R_2 = a skeleton of the non-nucleotide monomeric unit;

(b)  and

are first coupling groups in which:

X_2 = halogen or substituted amino

X_4 = halogen; amino; or O^-

R_3 = alkyl; alkoxy; or phenoxy

R_5 = alkyl; alkoxy; or aryloxy or may be H only if X4 = O^-

(c) R_1 -X_1 - is the protected second coupling group in which:

X_1 = O; S; NH; or HN=N-

R_1 = the protecting group cleavable under coupling group deprotecting conditions to recover the second coupling group H-X_1-;

(d) n is an integer;

(e) X_3-R_4- is the ligand.

11. (amended) A reagent as defined in claim 10 wherein:

X_2 = Cl; or secondary amino

R_2 = Chlorophenoxy;methoxy;ethoxy; or beta-cyanoethoxy

X4 = Cl; secondary amino; or O^- when R_5 = H

R5 = methoxy; ethoxy; monochlorophenoxy; or beta-cyanoethoxy; or may be H only if X4 = O^-

12. A reagent as defined in claim 11 wherein the ligand is a protected linking arm in which X3 is an alkylamino linked to R2 by a C- and to R4 by N-, and in which R4 is selected from trifluoroacetyl and 9-fluoremyl-methoxycarbonyl.

13. A reagent as defined in claim 11 wherein R_2 has a secondary carbon linked to the -O-.

14. A reagent as defined in claim 10, wherein the secondary amino groups are selected from dialkylamino, and heterocyclic N-amines.

15. (amended) A reagent as defined in claims 10, 11, or 14, wherein R_1 = triphenylmethyl or an alkoxy derivative thereof.

16. (amended) A reagent as defined in claim 10, 11 or 14, wherein X_1 = O and R_1 = triphenylmethyl or an alkoxy derivative thereof.

17. A reagent as defined in claim 10, 11 or 14 wherein X_1 = O and R_1 = dimethoxytriphenylmethyl.

18. (amended) A method of preparing a substituted nucleotide from a reagent having:

(a) a non-nucleotide monomeric unit; and

(b) a ligand linked to the non-nucleotide monomeric unit, and selected from a side arm with an attached chemical moiety, and from a linking arm to which a chemical moiety can be linked under non-adverse conditions; the method comprising coupling the non-nucleotide monomeric unit under non-adverse conditions, to a nucleotide monomeric unit and to one of a second monomeric unit and a solid support.

19. (amended) A method of preparing a substituted nucleotide from a reagent having:

(i) a non-nucleotide monomeric unit having a ligand, selected from a side arm with an attached chemical moiety and from a protected linking arm to which a chemical moiety can be linked; and

(ii) a first coupling group and a protected second coupling group linked to the non-nucleotide monomeric unit;

the method comprising, under non-adverse conditions:

(a) first coupling the non-nucleotide monomeric unit to a first additional monomeric unit through the first coupling group;

(b) then deprotecting the second coupling group;

(c) then coupling the non-nucleotide monomeric unit through the second coupling group, to a second additional monomeric unit;

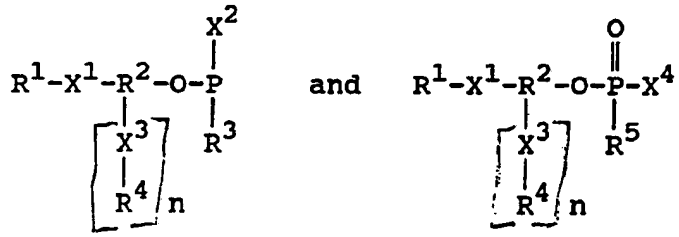
at least one of the first and second monomeric units being a nucleotide monomeric unit while the other one is selected from a monomeric unit and a solid support.

20. A method as defined in claim 18 or 19, wherein both the first and second additional monomeric units are nucleotide monomeric units.

21. A method as defined in claim 18 wherein the first and second coupling groups, are capable of coupling the non-nucleotide monomeric unit to a 5' hydroxyl and a 3' phosphate of a nucleotide monomeric unit, respectively.

22. A method as defined in claim 19 wherein the non-nucleotide monomeric unit has an acyclic skeleton to respective ends of which the first and second coupling groups are linked.

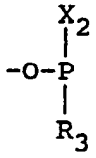
23. (amended) A method as defined in claim 19 wherein the reagent is selected from one of the compounds having the formula:



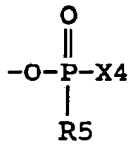
and

wherein:

(a) R_2 = a skeleton of the non-nucleotide monomeric unit;



and



are first coupling groups in which:

X_2 = halogen or substituted amino

X4 = halogen; amino; or O^-

R_3 = alkyl; alkoxy; or phenoxy

R5 = alkyl; alkoxy; or phenoxy; or may be H only if X4 = O^-

(c) R_1-X_1- is the protected second coupling group in which:

X_1 = O; S; NH; or HN=N-

R_1 = the protecting group cleavable under coupling group deprotecting conditions to recover the second coupling group H- X_1-;

(d) n is an integer;

(e) X_3-R_4- is the ligand

24. A method as defined in claim 23, wherein said ligand is selected from a label, an intercalator, a metal chelator, and from a linking functional group which can be linked to one of a label, an intercalator, and a metal chelator.

25. (amended) A method as defined in claim 24 wherein:

X_2 = Cl; or secondary amino

R_3 = chlorophenoxy; methoxy; ethoxy; or beta-cyanoethoxy

X4 = Cl; secondary amino; or O^- when R_5= H

R5 = methoxy; ethoxy; monochloophenoxy; beta-cyanoethoxy; or may be H only if X4 = O^-

26. A method as defined in claim 24 wherein the secondary amino groups of the reagent are selected from dialkylamino and heterocyclic N-amines.

27. A method as defined in claim 23 or 26 wherein R_1 of the reagent is triphenylmethyl or an alkoxy derivative thereof.

28. A method as defined in claim 23 or 26 wherein the reagent R_1 = dimethoxytripheynlmethyl.

29. A method as defined in claim 23 or 26 wherein the reagent has X_1 = O and R_1 = dimethoxytriphenylmethyl.

30. A method as defined in any of claims 23 to 26 additionally comprising coupling further monomeric units to the coupled nucleotide/non-nucleotide polymer, to produce a polymer with a nucleotide sequence which is complementary to at least a portion of a target nucleotide sequence, but which complementary nucleotide sequence has at least one non-nucleotide monomeric unit disposed therein, then hybridizing the nucleotide/non-nucleotide polymer sequence with the target sequence.

31. (amended) A composition containing a single sequence nucleotide/non-nucleotide polymer as the majority

of all nucleotide/non-nucleotide polymers present, the single nucleotide/non-nucleotide polymer comprising:

(a) a non-nucleotide monomeric unit having linked thereto a ligand selected from a side arm with an attached chemical moiety, and from a linking arm which can be linked to a chemical moiety under non-adverse conditions;

(b) a first nucleotide monomeric unit coupled to the non-nucleotide monomeric unit; and

(c) an additional monomeric unit coupled to the non-nucleotide monomeric unit.

32. A reagent as defined in claim 31, wherein said ligand is selected from a label, an intercalator, a metal chelator, and from a linking functional group which can be linked to one of a label, an intercalator and a metal chelator.

33. (amended) A composition as defined in claim 31 or 32 wherein the additional unit is a nucleotide monomeric unit.

34. (amended) A composition as defined in claim 31 or 32 wherein the additional monomeric unit is a non-nucleotide monomeric unit, which is coupled to another non-nucleotide monomeric unit.

35. (amended) A composition containing a nucleotide non-nucleotide polymer comprising:

(a) a non-nucleotide monomeric unit, having linked thereto a ligand selected from a side arm with an attached chemical moiety linked to the skeleton through a saturated linker group, and from a saturated linking arm which can be linked to a chemical moiety under non-adverse conditions;

(b) a first nucleotide monomeric unit coupled to the non-nucleotide monomeric unit; and

(c) an additional monomeric unit coupled to the non-nucleotide monomeric unit.

36. A composition as defined in claim 35, wherein said ligand is selected from a label, an intercalator, a metal chelator, and from a saturated linking arm which can be linked to either of a label and an intercalator.

37. A composition as defined in claim 36 wherein the ligand group is the saturated linking arm.

38. A composition as defined in claim 34 wherein the non-nucleotide monomeric unit has a skeleton to the ends of which are linked the coupling groups, and wherein the ligand comprises a label linked to the skeleton through

the linking group which is selected from an alkylamino and alkylthio.

39. A composition as defined in claim 37 wherein the linking arm is selected from an alkylamino and an alkythio.

40. A composition as defined in claim 35, 37 or 39 wherein the non-nucleotide monomeric unit has a skeleton to the ends of which are linked the coupling groups, the skeleton being an acyclic hydrocarbon chain having from 1 to 20 carbon atoms.

41. A composition as defined in claim 35 wherein the label is a chemiluminescent acridinium ester.

42. A composition as defined in claim 36 wherein the intercalator is an acridinium ester.

43. (amended) A composition as defined in claim 35 wherein the label is selected from biotin, fluorescein, dinitrobenzene, rhodamine, and Texas Red.

44. A composition as defined in claim 35 wherein the intercalator is selected from psoralen, acridine, acridinium salts, acriflavins, and ethidium.

45. A polymeric probe comprising a plurality of nucleotide monomeric units, and at least one acridinium ester moiety linked to a corresponding monomeric unit of the probe.

46. A probe as defined in claim 45 wherein the acridinium ester label is a chemiluminescent acridinium ester label.

47. A method of making a probe, comprising linking at least one acridinium ester moiety to a corresponding monomeric unit of a polymer having a plurality of nucleotide monomeric units.

48. A method as defined in claim 47 wherein the acridinium ester label is a chemiluminescent acridinium ester label.

49. (new) A reagent as defined in claims 10, 11, 24, 25, 31, or 35, wherein the ligand is a protected linking arm in which R_4 is the linking arm and X_3 is the protecting group.

50. (new) A reagent as defined in claims 10, 11, 24, 25, 31, or 35, wherein the ligand is a protected linker arm in which X_3 is an alkylamino linking arm linked

to R_2 by a C- and to R_4 by N-, and R_4 is the protecting group.